

Memo/Reply From
JOSHUA LEDERBERG

TO:

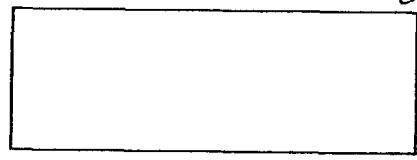
Bill Decker
OCT 3 1979

Bruce Ames

*Re NAS/Env. Inquiry
answered.*

*I hope this is helpful.
Much help was given by
Vicki McElheney and
(indirectly) Dan Roseland*

*Sincerely,
Joshua*



Joshua Lederberg
President
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☐ ORIG.
☐ COPY RETAINED

- REPLY FORM -



1980

NOMINATIONS FOR THE
1979 GENERAL MOTORS CANCER RESEARCH PRIZES

Please Specify:

☐ Sloan Prize☐ Kettering Prize☒ Mott Prize

NAME OF CANDIDATE: Bruce Ames

ADDRESS: Department of Biochemistry
University of California, Berkeley
Berkeley, California 94720

ACADEMIC OR INSTITUTIONAL AFFILIATION AND TITLE:

University of California, Berkeley. Professor.

THE DISCOVERY OR CONTRIBUTION:

The Ames Test for Environmental Carcinogens

BRIEF DESCRIPTION OF THE DISCOVERY OR CONTRIBUTION (include pertinent reference material):

Ames has developed a rapid, sensitive and economical method for detecting environmental carcinogens. The test, already in widespread use, has made it possible to detect carcinogens with a speed and economy never achieved previously. As a result testing of compounds has demonstrated the presence of carcinogens in materials and sources previously unsuspected as sources of cancer. Furthermore the theory on which these tests have been based — that carcinogens are mutagens — has made a major contribution to the theory of carcinogenesis. Finally, the simplicity and accuracy of the test allows pre-testing of chemicals contemplated for widespread use and thus should have a major preventative value in the future.

This work has appeared in a number of scientific papers cited in the bibliography of which the most representative are:

Ames, *et al.*, "An improved bacterial test system for the detection and classification of mutagens and carcinogens," *Proc. Nat. Acad. Sci. USA* 70, 782 (1973); "Carcinogens are mutagens: A simple test system combining liver homogenates for activation and bacteria for detection," *PNAS* 70, 2281 (1973); "Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test," *Mutation Res.* 31, 347 (1975). McCann, *et al.*, "Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals," Part I in *PNAS* 72, 5135 (1975); Part II in *PNAS* 73, 950 (1976).

NAME OF PROPOSER: Daniel E. Koshland, Jr.

ADDRESS: Department of Biochemistry
University of California, Berkeley
Berkeley, California 94720

INSTITUTIONAL AFFILIATION AND TITLE:

University of California, Berkeley. Professor.

SIGNATURE

DATE

(Over)

CURRICULUM VITAE

NAME: Bruce N. Ames

DATE OF BIRTH: 16 December 1928

PLACE OF BIRTH: New York, New York

MARITAL STATUS: Married to Dr. Giovanna Ferro-Luzzi Ames
Two children

EDUCATION: Cornell University, B.A.
Chemistry Major, Biology Minor 1946-1950
California Institute of Technology, Ph.D.
Biochemistry Major with Prof. H.K. Mitchell;
Chemistry and Genetics Minors 1950-1953

PROFESSIONAL
EXPERIENCE: Postdoctoral Fellow (U.S.P.H.S.) at N.I.H.
with Dr. B.L. Horecker 1953-1954
Biochemist at the National Institutes of Health 1954-1960
Sabbatical year as N.S.F. Senior Fellow in
Laboratories of F.H.C. Crick in Cambridge,
England and F. Jacob in Paris, France 1961
Chief, Section of Microbial Genetics, Lab. of
Molecular Biology, N.I.A.M.D., N.I.H. 1962-1967
Professor of Biochemistry, Dept. of Biochemistry
University of California at Berkeley 94720
(415) 642-5165 1968-present

PROFESSIONAL
SOCIETIES: American Chemical Society
American Society of Biological Chemists
Genetics Society of America
American Society for Microbiology
Environmental Mutagen Society
American Association for Cancer Research
Society of Toxicology

HONORS: American Academy of Arts and Sciences 1970
National Academy of Sciences 1972

AWARDS: Eli Lilly Award of the American Chemical Society 1964
Arthur Flemming Award (as outstanding young
Government Employee) 1966
Lewis Rosenstiel Award 1976
FASEB/3M Award for Research in Life Sciences 1976
E.R.D.A. Distinguished Associate Award 1976
Environmental Mutagen Society Award 1977
Cal. Tech. Distinguished Alumni Award 1977
Simon Shubitz Cancer Prize 1978
Felix Wankel Research Award 1978
John Scott Medal 1979

(continued)

SERVICE ON BOARDS
AND COMMITTEES:

Program Committee of the Am. Soc. Biol. Chem.	1963-1967
Editorial Board, <u>Arch. of Biochem. and Biophys.</u>	1964-1969
Member of National Research Council (representative of Genetics Society)	1964-1969
Editorial Board, <u>Journal of Biological Chemistry</u>	1965-1971
Nominating Committee of the American Society of Biological Chemists	1967-1969
Governing Council, Environmental Mutagen Society	1971-1975
Advisory Committee, Earl Warren Legal Institute	1971-1975
Nominating Committee of the Genetics Society	1971
Organizer, 1st International Conference on Environmental Mutagens, Asilomar, California	1973
Consultative Panel on Hazards of Chemical Pesticides, Nat. Res. Council, Nat. Acad. Sci.	1974
Subcluster on Environmental Health and Toxicology of President's Biomedical Research Panel	1975
National Cancer Advisory Board	1976-present
Search Committee for director of N.C.I.	1977

RESEARCH INTERESTS:

Detection of environmental chemicals causing damage to DNA, chemical mutagenesis and carcinogenesis, biochemical genetics of bacteria, regulation of gene expression, operons, histidine biosynthesis.

More Extended Description of the Contributions of

PROFESSOR BRUCE N. AMES

Ames is one of the pioneers of molecular biology. His early work was concerned with the genetic control of gene expression in bacteria and his recent research with the demonstration that chemicals causing cancer in mammals are strongly correlated with mutagens in microorganisms.

Ames has made important contributions in an extremely wide range of different fields: physical chemistry, analytical chemistry, mutational theory, metabolism, enzymology, microbiology, virology, evolution, bacterial genetics, gene regulation, drug design, active transport, antibiotics, tRNA, protein synthesis, pharmacology, chemical carcinogenesis, and environmental toxicology. He is one of the most cited scientists in the world for the period 1961-1976 according to the Science Citation Index (Current Contents, July 10, 1978), and in the last two years his recent work on the relation between mutagens and carcinogens and on his mutagen test system have become among the most cited papers in the scientific literature (Science Citation Index, 1977, 1978).

In recognition of the contributions to science of all of this work, Ames was elected to the American Academy of Arts and Sciences in 1971 and to the National Academy of Sciences in 1972, and he has also received numerous awards which are listed in the attached curriculum vitae.

Environmental Mutagens and Carcinogens

In 1964, Ames became interested in the problem of whether environmental chemicals might be mutating the human gene pool and contributing to the high

rate of genetic defects in human births. As a side project in his laboratory he decided to develop a simple method for the detection of mutagens so that environmental chemicals could be screened for mutagenicity. The test he has developed over the last 14 years is a rapid, sensitive, and economical method (using Salmonella bacteria and mammalian microsomal enzymes) for detecting environmental mutagens, and it has become extremely widely used. One of the main results to come out of his work, in addition to the test system itself, is that almost all of the carcinogens he has examined are detected as mutagens by the test system whereas very few non-carcinogens are mutagenic. As a result of this work, he has supported the theory that almost all known chemical carcinogens cause cancer through somatic mutation and this and other work have led to a revitalization of this theory. His work has made a major contribution to the theory of mutagenesis, the theory of cancer, and has caused a revolution in the area of toxicology concerned with mutagens and carcinogens.

Theory of Mutagens

Ames and his group's first studies on mutagenesis involved a study of a large number of mutants in one of the genes of histidine biosynthesis. They developed methods for classifying the mutations into missense, nonsense, and frameshift types. One of the conclusions from this work was that most mutations resulted in proteins that were functional and that therefore mutation rates were about ten times higher than generally suspected. They did this by estimating the ratio of missense to nonsense mutants. This work, and other work with Hartman on the genetics of the histidine operon, was later extremely useful in the work on developing the tester strains for the detection of mutagens.

He has also made a major contribution to mutagenic theory in the area of frameshift mutations. In an extremely influential paper in the Cold Spring Harbor Symposium in 1966, and in later work, he and Whitfield showed that a class of polar mutations in the histidine operon were frameshift mutations. They showed that these were not reverted by the usual alkylating agents that reverted missense or nonsense mutations and they characterized an unusual class of molecules, the ICR compounds, as frameshift mutagens. The ICR compounds were the first frameshift mutagens described for bacteria. (Crick and Brenner, et al. had done some of the early work on the genetic code on frameshift mutants in phage and had used the mutagen proflavin, but this mutagen wasn't very effective on bacteria.) The interesting aspects of the structure of the ICR chemicals that Ames and Whitfield pointed out (they examined a large number of different structures that had been made by High Creech, et al.) was that they consisted of a bifunctional agent, a flat aromatic acridine ring that was capable of a stacking interaction with DNA and a half mustard side chain that could alkylate guanine. Ever since this work these agents have been widely used in both bacteria and in animal cells for causing frameshift mutations. Ames and his colleagues then used the ICR mutagens to develop tester strains sensitive to frameshift mutagens. These were later shown to have long repetitive sequences in the DNA: one of their tester strains has a repetitive -GGGGG- sequence across from a -CCCCC- sequence and another has an alternating -GCGCGCGC- sequence. Ames and his students found mutagens specific for each type of sequence. These strains were crucial in showing in later years that the active form of one class of carcinogens were frameshift mutagens (these include polycyclic carcinogens such as benzo(a)pyrene

and aromatic amines such as benzidine and acetylamino fluorene). Ames and his colleagues made the point that the active forms of certain carcinogens were frameshift mutagens and that the structural principles were similar to the ICR compounds in that the molecules consisted of an aromatic ring system capable of a stacking interaction with DNA and an electrophilic side chain capable of covalent interaction with DNA.

The Ames Test

The Ames test system uses a set of histidine-requiring mutants of Salmonella typhimurium coupled with a mammalian liver homogenate (rat or human autopsy) for carcinogen and mutagen activation. All of the ingredients are added to the petri plates together and the revertant colonies are scored after two days. The principle of the tester strains is to use mutants of a known type of DNA damage (base pair substitutions and the various kinds of frameshift mutations) for detecting mutations by the sensitive and convenient back mutation test. He has screened hundreds of mutations to obtain those in the tester strains which are the most sensitive to the various kinds of mutagens. In the case of the frameshift mutations these have turned out to be long strings of repetitive bases (e.g. CGCGCGCG or GGGGGGG) which are "hot spots" for frameshift mutagenesis by particular compounds. (Some of the DNA sequences have been determined by doing protein chemistry on +- revertants of the frameshift mutations.) He has further improved the sensitivity of the tester strains by up to 100-fold by eliminating the main DNA excision repair system in the strains, and has made another improvement in sensitivity of up to 100-fold by introducing a mutation that strips off the lipopolysaccharide in the bacteria, which acts as a barrier to the larger mutagens dissolving in the bacterial membrane.

Initially only some of the known chemical carcinogens caused growth of revertant colonies but Ames and his colleagues improved the sensitivity of his system step by step until he was able to prove a strong correlation between carcinogenicity and mutation. The fourth improvement (after mutagenic hot spots, defective repair, and loss of the lipopolysaccharide barrier) was to increase mutagenesis in his tester strains by certain important carcinogens such as aflatoxin B₁, by incorporating a plasmid which appears to channel DNA damage down an error-prone repair pathway (SOS repair).

Ames and his students had made one more improvement in the system which proved decisive. It had been discovered by the Millers and other workers that in mammals many compounds are non-carcinogenic as such, but are converted into carcinogens by the oxidizing enzymes such as cytochrome P450 present in microsomes of the liver and other tissues. Following observations by several workers who had incubated microsomes with a particular chemical to obtain active forms, Ames decided to mimic this conversion in the mammal by adding rat (or human liver) homogenate together with a NADPH generating system to his petri plates. The liver homogenate could be frozen successfully for long periods of time and can then be thawed and added directly to the petri plate with the tester strain and the carcinogen. Thus he could activate a wide variety of carcinogens into their active (and mutagenic) forms by this simple "first approximation" of a rat.

In addition to developing the mutagenicity test system, Ames and his students spent several years of optimizing, standardizing, and simplifying the method, and their paper on the test method is becoming one of the most highly cited scientific papers in the world (Science Citation Index, 1977, 1978).

Validation: Almost all Carcinogens Tested can be Detected as Mutagens

Joyce McCann, a postdoctoral student, and Ames have validated the test in a monumental study in which they examined about 300 carcinogens and non-carcinogens in the system. They have shown a remarkable correspondence between carcinogenesis in mammals and mutagenesis in Salmonella. About 90% (157/175) of the carcinogens were mutagenic in the test including almost all of the known human carcinogens that were tested. Despite the severe limitations inherent in defining non-carcinogenicity, few "non-carcinogens" showed any degree of mutagenicity. This validation involved a massive amount of work in evaluating carcinogenicity tests, in assembling the large numbers of chemicals of sufficient purity, and doing the test itself. Two other groups have independently validated the Ames test system recently. The National Cancer Center Research Institute in Japan and Imperial Chemical Industries (ICI), one of the largest chemical companies in Europe, have examined over 100 chemicals each and both found about the same 90% accuracy rate in detecting carcinogens as mutagens in the test.

Only about a dozen or so organic chemicals are currently known which cause cancer in people (because of the difficulties in human epidemiology when one is dealing with a 20-year lag) and almost all of these are mutagens in the Salmonella test system.

A few important classes of carcinogens, however, are not well detected as mutagens and Dr. Ames' present work on these classes is bearing fruit. Some recent (unpublished) work has shown that several of these classes can now be detected by understanding some principles and making some sophisticated, but simple, additions to the test. One of these advances is the development of an

effective model for the metabolism of human gut bacteria that can be added to the test system in a simple way. This has enabled the test to detect carcinogenic glycosides of mutagens that were not previously detected.

Complex Mixtures: Cigarette Smoke

The economy of the bacterial/mammalian-microsomal assay suggests its usefulness as a tool in rapidly obtaining information about the potential mutagenic/carcinogenic activity of uncharacterized compounds in complex mixtures. Ames and his students made a detailed study of the mutagenic activity of cigarette smoke condensate and 12 standard smoke condensate fractions. Whole condensates were shown to contain compounds that were frameshift mutagens after activation by rat liver or lung microsomal preparations. The condensate from less than 0.01 cigarette could easily be detected: about 29,000 revertant colonies were obtained per cigarette. They concluded that microsomal enzymes in the lung may be an important factor in the biological activity of cigarette smoke. They found activity in several of the condensate fractions. It is clear that this assay can be used as a quick bioassay for isolating the various compounds in the condensate that are mutagenic and these will presumably include the active carcinogens. Cigarette companies are now doing this.

Dr. Ames and his colleagues have recently developed a simple method for assaying the mutagenicity of human urine in their test system. This is an improvement on their earlier method for assaying urine for mutagenicity. Using this new method, they have shown that cigarette smokers have significant amounts of mutagens in their urine while non-smokers do not. This method is

now being used by a large number of laboratories for assaying human urine, water supplies, and other aqueous fluids.

Applications of the Ames Test

The Ames test is rapidly starting to play a central role in a long-term toxic substances monitoring program aimed at identifying and minimizing human exposure to environmental carcinogens and mutagens. It is a complement to the traditional animal carcinogenicity tests (which take 2-3 years and cost about \$200,000) as it can be used in a variety of ways not feasible with animal tests. It is currently being used in over 2,000 laboratories and most of the major chemical and industrial companies in the world, and a flood of papers are appearing using it for many of the following applications:

1. Chemical, drug, and industrial companies can now afford to do routing tests of new compounds at an early stage of development so that mutagens can be identified and this information taken into consideration before there is a large vested interest in the compound.
2. If a useful compound, such as a drug or pesticide, is mutagenic, a variety of derivatives can often be synthesized, and often a non-mutagenic form can be substituted.
3. Many companies are finding that the mutagenicity of a chemicals is due to a trace of impurity (which can be removed) and this can save a useful chemical. Ames and his students have published an interesting paper pointing out many cases of mutagenic impurities and their impact on the chemical industry.
4. Complex mixtures, or natural products with carcinogenic activity, can be investigated using the test as a bioassay for identifying the mutagenic

ingredients. This is being done in a very wide variety of areas such as automobile exhaust, cigarette smoke, and plant carcinogens (such as bracken fern).

5. Human feces and urine can be examined to see if ingested products or drugs are giving rise to mutagens.

6. The variety of substances that humans are exposed to, both pure chemicals and mixtures, are being assayed for mutagenicity by thousands of laboratories.

7. The active metabolic forms of chemical carcinogens and their metabolic routes can be determined using the test as a bioassay.

8. The test system is useful in clarifying basic mechanisms of mutagenesis: e.g., frameshift mutagenesis where chemicals prefer particular base-pair sequences and the clarification of the role of different DNA repair system.

9. The test is useful in setting priorities for further toxicological testing such as carcinogenesis testing in animals or human epidemiology.

Other Short-term tests

Since Ames' development of his Salmonella/liver test and his demonstration that almost all carcinogens are mutagens, there has been a tremendous resurgence of interest in short-term test systems for measuring mutagenicity. Many such systems have been developed and are starting to be validated, including the use of animal cells in tissue culture. The use of a battery of these tests is clearly a powerful new toxicological tool. The Salmonella test still appears to be the most validated, accurate, simplest, and inexpensive overall, and is in use in by far the widest number of laboratories.

Hair Dyes and Tris

In addition to the theoretical and methodological advances coming out of the Ames laboratory, he and his students, in a series of important papers, have been among the leading workers in utilizing mutagenicity testing for illuminating what may be major environmental hazards. Ames had discovered that almost all hair dyes on the market were mutagenic in his system. In an influential paper that has been held up as a model of thoroughness and breadth in toxicology, he investigated the individual hair dye components, the peroxide oxidation products, and the general area of hair dye toxicology. This paper was one of the major causes of a congressional investigation of the regulation of cosmetics and many new investigations of cosmetic toxicology. A number of the hair dye ingredients Ames found to be mutagenic have now been tested by N.C.I. and have been shown to cause cancer when fed to rats and mice. They have also been shown to be mutagens in a variety of other short-term tests. In addition, several epidemiological studies suggest that beauticians have a higher rate of cancer than expected. A new study that was recently published by Shore's group at N.Y.U. suggests that women who have dyed their hair for a long time may have a markedly increased rate of breast cancer. More epidemiology will certainly be done to see whether these studies can be substantiated, as an appreciable percentage of the women in the U.S. dye their hair.

Fifty million children have been exposed to the flame-retardant Tris which was added to polyester sleepwear even though only minimal toxicology was done on it before use. Ames became interested in this substance when he went out to buy his children some pajamas. After some conversations with Prival at EPA (who also had become interested in the problem), both groups showed that

Tris and its metabolites were strong mutagens in the Ames test. Blum and Ames, in a classic paper on toxicology that appeared as a lead article in Science, discussed the general problem of the hazards of flame retardants, the alternatives, the problems of skin absorption, and why they thought that the use of Tris was likely to be quite hazardous as a potential carcinogen and mutagen. Their work was later substantiated by many other short-term tests which showed that Tris was mutagenic, by the cancer tests at N.C.I. which showed that Tris was a strong carcinogen in both rats and mice, by skin painting studies in animals which showed that Tris caused cancer, and by other animal studies showing Tris caused testicular atrophy and sterility. Recent work by Ames and his collaborators has shown that Tris is indeed absorbed through the skin of children wearing Tris-treated pajamas, as the mutagenic metabolite, dibromopropanol, could be detected in the children's urine.

Widespread Use of the Ames Test

A very large number of papers have appeared using the Ames Salmonella test as a tool in investigating mutagens (and presumptive carcinogens) in our world, both natural and man-made. This can be seen by the list from the Environmental Mutagen Information Center at Oak Ridge, Tennessee or by looking up the Ames, McCann and Yamasaki Methods paper in the Science Citation Index. These thousands of papers cover areas as diverse as pesticides, water contaminants, air contaminants, cigarette smoke ingredients, diesel fuel and car exhaust mutagens, green plant products, chlorinated water, paper bleaching effluents, industrial chemicals, drugs, plastic ingredients, and flame retardants.

It seems clear that even though only a small percentage of chemicals in general are mutagens we are exposed to a wide variety of mutagens from many sources, and that the short-term tests, among which Salmonella is preeminent, will play a crucial role in identifying these many substances.

Two test cases of major importance are in progress using the Salmonella test as a bioassay for natural carcinogens. Dr. W. R. Bruce and his colleagues in Toronto have found a considerable amount of a powerful mutagen in human feces. It appears to be a nitrosamine formed from a component of dietary fat and could be a major cause of colon and breast cancer, two common cancer types associated with high fat intake. Dr. Bruce is identifying its chemical structure using Salmonella mutagenicity as a bioassay. He is also finding that high vitamin C or vitamin E intake lowers the amount of the mutagen. In another instance, Dr. Sugimura and his colleagues in Tokyo have discovered that when fish are broiled (a common practice in Japan), mutagenic chemicals are formed. Using the Ames Salmonella test as a bioassay, they have found that the broiling protein produces mutagens and that broiling tryptophan (a component of protein) produces potent mutagens. They have identified one active mutagen chemically and shown that it is also very active in another short-term assay (transformation) using animal cells. They are currently doing an animal cancer test on the substance. An animal cancer test could never have been used as the bioassay for identifying the chemical because of the time involved. Thus, based on the mutagenic activity for Salmonella, it appears that broiling food so that burnt protein is formed may contribute a fairly substantial dose of mutagens to our diet. Eating a barbecued fish

or steak could well be equivalent to smoking a few packs of cigarettes in terms of the amount of mutagen that enters our body.

Carcinogenic Potency

Ames and his students will shortly publish an exhaustive study which they have conducted over the last two years on carcinogenic potency. -With the help of Richard Peto in England, they devised a method for calculating the potency of carcinogens in lifetime feeding and inhalation studies. They then assembled a computer data base from which they have calculated potency for a very high percentage of the animal cancer tests in the world's literature and for the 200 chemicals that N.C.I. has just tested. This analysis has illuminated many aspects of animal cancer tests: the range of potency of carcinogens (currently about 100-million-fold: from 50 ng/kg/day to 5 g/kg/day to give half of the animals cancer); the degree of similarity of cancer tests on different sexes, strains, and species; the degree of reproducibility of cancer tests on the same organism; a method for quantitatively measuring the thoroughness of negative cancer tests; the range of potency; and the allowed level of industrial carcinogens or body residues versus the level to which humans are being exposed. The study should become a landmark as a more rational way of setting priorities for dealing with carcinogens. Ames has also been exploring how the short-term tests may contribute to setting priorities for the 50,000 or so chemicals used in commerce that have not been tested in animal cancer tests and for the very large number of mutagens in the natural world that have not been tested for carcinogenicity. He believes that the short-term tests may be able to provide us with a rough idea of potency, given

the enormous range of potency of carcinogens, and thus help in the monumental problem of setting priorities for the many substances that damage our DNA.

(The articles with numbers followed by the letter "a" are from the Ames lab, but of which Dr. Ames is not a coauthor.)

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